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KRAS/NRAS MUTATIONAL ANALYSIS AND CLINICOPATHOLOGIC CORRELATION OF COLORECTAL CARCINOMA: A SINGLE INSTITUTE EXPERIENCE FROM TURKEY

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ABSTRACT

Background: Colorectal carcinoma (CRC) is the second most frequent cause of cancer-related death for both sexes. Clinical and pathological correlates of Kirsten rat sarcoma viral oncogene/neuroblastoma ras viral oncogene (KRAS/NRAS) mutant tumors are important for therapy response, especially in metastatic CRCs. The aim of this study is to determine the frequency of KRAS/NRAS mutations and investigate the clinicopathologic characteristics of KRAS/NRAS mutant and rat sarcoma (RAS)-wild type CRCs. **Methods:** Pathology archives were searched for CRCs between 2014 and 2015, retrospectively. We reevaluated tumor slides for Crohn-like infiltrate, tumor infiltrating lymphocytes, tumor budding, presence of mucinous component, and signet-ring cell morphology. Tumor grade, depth of invasion, lymph node metastases, distant metastases, lymphovascular and perineural invasion, polyp type, and DNA mismatch repair status were derived from their pathology reports. Formalin-fixed, paraffin-embedded tissues were examined for KRAS/NRAS mutation status. Mutation status and their clinicopathologic correlates were evaluated. **Results:** KRAS and NRAS mutations were detected in 43.6% and 10% of the CRCs, respectively. KRAS mutations were associated with multiple organ metastasis and CRCs with solid growth pattern did not harbor RAS mutations. Well differentiated CRCs were more common in NRAS mutant and RAS-wild type CRCs in comparison to RAS mutant tumors. NRAS mutant CRCs were more frequent in the left colon and rectum. **Conclusion:** In this study, we identified that KRAS mutations were associated with multiple organ metastasis and CRCs with solid growth pattern did not harbor RAS mutations. Also, NRAS mutant tumors were more common in the left colon and rectum concordant with the previous studies.

Keywords: Colorectal Carcinoma, Metastatic Colorectal Carcinoma, KRAS, NRAS, RAS.

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INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer in men, the second most common in women, and for both sexes, the second most frequent cause of cancer-related death worldwide [1]. Despite increasing awareness of this cancer and screening programs, twenty-two percent of the patients have concurrent metastases at the time of diagnosis for which 5-year survival rate is 13.3% [2]. Approximately last two decades, the important part of CRC research has engaged in targeted therapy especially for metastatic CRC (mCRC) patients for extending survival rate [3].

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Monoclonal antibodies (i.e. cetuximab and panitumumab) targeting epidermal growth factor receptor (EGFR) introduced in the treatment of mCRC in combination with oxaliplatin- or irinotecan-based cytotoxic chemotherapy which improved the survival rates. However; mCRCs harboring RAS mutations identified as unresponsive to anti-EGFR therapy, as mutant RAS proteins are constitutively active downstream to EGFR [4, 5]. Currently, National Comprehensive Cancer Network guidelines recommend KRAS, NRAS, B-type Raf proto-oncogene (BRAF) mutational analysis for anti-EGFR therapy candidates in mCRCs [6].
Approximately 40% of the CRCs harbor KRAS, 5% NRAS, and 10% BRAF mutations [8-15]. Thus; clinical and pathological correlates of KRAS/NRAS mutant tumors are important for the practicing pathologist and oncologist as activating RAS mutations constitute the most common genetic alteration and predict therapy response to tyrosine kinase inhibitors in mCRCs. Prior studies associated certain clinical (age, gender, family history, cigarette smoking) and pathologic findings (tumor site, differentiation, lymphovascular invasion, tumor budding, lymph node, and distant metastases) with RAS mutations [7-10]. However; results are conflicting among different researchers. Therefore, we aimed to determine the KRAS and NRAS mutation frequency in metastatic and non-metastatic CRCs in a single institute in Turkey; in addition, evaluated the association of RAS mutation status with a wide spectrum of patient and tumor-related factors.

MATERIAL AND METHODS

Patient selection

Pathology archives were searched for the surgically resected CRCs between February 2014 and November 2015. Forty-two consecutive mCRCs with RAS mutational profiling as part of their standard care and 68 non-mCRCs with available blocks/slides were included in the study. Age, gender, family history of colon carcinoma, and cigarette smoking status of 110 patients were retrieved from the oncology files. Our patients followed up from oncology were called to the hospital and their consents were obtained for the study.

Histopathologic evaluation

Tumor slides were reevaluated for Crohn-like infiltrate, tumor infiltrating lymphocytes (TIL), tumor budding, presence of mucinous component, and signet-ring cell morphology. Tumor budding scored in hematoxylin-eosin stained slides as previously described [11]. The rate of the mucinous component was graded as >10% and <10%. All CRCs in the cohort were adenocarcinomas. Other histopathologic features such as tumor grade, depth of invasion, lymph node metastases, distant metastases, lymphovascular and perineural invasion, polyp type, and DNA mismatch repair (MMR) status were derived from the initial pathology reports.

Molecular testing

Tumor slides were reviewed and blocks with the highest tumor ratio were selected for DNA isolation with AmoyDx® (Amoy Diagnostics) KRAS and NRAS mutation kits. KRAS codon 12 (G12D, G12A, G12V, G12S, G12R, G12C), 13 (G13D, G13C), 59 (A59T), 61 (Q61K, Q61L, Q61R, Q61R, Q61H), 117 (K117N), 146 (A146T, A146V, A146P) and NRAS codon 12 (G12D, G12S, G12C, G12V, G12A), 13 (G13D, G13R, G13V), 59 (A59D), 61 (Q61R, Q61K, Q61L, Q61H), 117 (K117N), 146 (A146T) mutations were evaluated. Samples were analyzed in Cobas 4800 Diagnostic System® (Roche Diagnostics) according to the manufacturer's instructions. All experiments were performed with mycoplasma-free cells.

Statistical analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. To compare qualitative variables, One-Way Anova, Tukey HSD, and Pearson Chi-squaretests were used to compare different mutational groups in relation to clinicopathologic features. P-values < 0.05 considered as statistically significant.

Ethical statement

The study was approved by Institutional Review Board, and all patients were given informed consent.

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RESULTS

Clinicopathologic features

Seventy-one (64.5%) patients were male and the median age was 66 (30-87) years. Sixty-six (60%) patients were former or current smokers and twelve (10.9%) revealed a family history of colon carcinoma. Forty-two (38.1%) patients had concurrent distant metastases. The liver was the most common metastatic site and 12 patients (11%) had metastatic disease to multiple sites. Thirty-six (32.7%) cases had a polyp in the resection specimen or prior polypectomy history. Of the 36 cases, 91.7% (n=33) was adenomatous and 8.3% (n=3) was serrated histology. MMR status evaluated in 29.2% (n=31) of the study cohort and 2.7% (n=3) of them recognized as MMR deficient. Tumor localization and other histopathologic features were summarized in **Table I**.

Table I: Tumor characteristics.

		N	%
Localization	Cecum	17	15.5
	Ascending colon	3	2.7
	Hepatic flexure	7	6.4
	Transverse colon	8	7.3
	Splenic flexure	3	2.7
	Descending colon	8	7.3
	Sigmoid colon	43	39.1
	Rectum	21	19.1
Differentiation	Well	13	11.8
	Moderately	84	76.4
	Poorly	13	11.8
	Undifferantiated	0	0
Lymphovascular invasion (+)		52	47.3
Perineural invasion (+)		37	33.6
Metastatic site	Liver	25	22.7
	Lung	3	2.7
	Periton	2	1.8
	Liver+lung	8	7.3
	Liver+bone	1	0.9
	Liver+lung+periton	2	0.9
	Liver+supraclavicular lymph node	1	0.9

Tumor budding found to be low score in 57.3% (n=63) of patients, intermediate in 33.6% (n=37), high in 8.2% (n=9), and in one case cannot be assessed due to the high lymphocytic infiltration in the invasion border. Crohn-like infiltrate was present in 27.3% (n=30), TIL in 0.9% (n=1), signet-ring cell morphology in 2.7% (n=3), solid growth pattern in 8.2% (n=9) of the cases. Mucinous component greater than 10% was observed in 21.8% (n=24) of the CRCs.

Genotyping results and correlation of RAS mutations with clinicopathologic features

Mutational analysis performed for 68 non-metastatic CRCs and the RAS status of 42 metastatic CRCs were derived from initial pathology reports. Overall RAS mutations were identified in 53.6% (n=59) of the 110 CRCs. KRAS and NRAS mutation frequency were 43.6% (n=48) and 10% (n=11), respectively. The most common mutation was KRAS exon 2 mutations and detected in 43 (72.9%) patients (**Table II**).

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Table II: Distribution of mutations.

	Exon	Codon (n,%)	Mutation (n)
KRAS (n=48, 81.4%)	2	12 (41, 69.5)	G12A (12)
			G12C (3)
			G12D (11)
			G12R (3)
			G12S (2)
			G12V (10)
		13 (2, 3.4)	G13C (1)
			G13D (1)
	3	61 (2, 3.4)	Q61K (1)
			Q61R (1)
	4	117 (2, 3.4)	K117N (2)
		146 (1, 1.7)	A146T (1)
NRAS (n=11, 28.6%)	2	12 (7, 11.9)	G12A (2)
			G12C (2)
			G12D (1)
			G12S (1)
			G12V (1)
		13 (1, 1.7)	G13V (1)
	3	61 (3, 5.1)	Q61K (1)
			Q61R (2)

There was no statistically significant association between RAS mutations, age, gender, cigarette smoking, and family history (p >0.05). NRAS mutant and RAS-wild type (RAS-wt) CRCs were more frequently well differentiated in comparison to KRAS mutant tumors (p=0.079). Tumors with solid growth pattern were all RAS-wt (p=0.003). Lymphovascular and perineural invasion, Crohn-like infiltration, mucinous component, and signet ring cell differentiation, and tumor budding were not associated with RAS mutational status (p >0.05). RAS mutation frequency was not different between metastatic and non-metastatic CRCs (p >0.05). Well differentiated CRCs were more common in NRAS mutant and RAS-wild type CRCs in comparison to RAS mutant tumors (p=0.079). NRAS mutant CRCs were more frequent in the left colon and rectum than the right colon. KRAS and NRAS mutant tumors were associated with multiple organ metastases in comparison to RAS-wt type tumors (p=0.055) (**Table III** and **Table IV**)

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Table III: Comparison of clinicopathologic features with mutational groups.

		KRAS	NRAS	RAS-wt	p-value
Age (years)	Min-max (median)	42-85 (66.5)	30-85 (61)	34-87 (66)	[c]0.353
	Mean, SD	65.40 +-10.06	59.73 +-16.88	64.94+-12.27	
Gender; n (%)	Male	33 (68.8)	8 (72.7)	30 (58.8)	[a]0.491
	Female	15 (31.3)	3 (27.3)	21 (41.2)	
T; n (%)	T1-2	4 (8.3)	2 (18.2)	8 (15.7)	[a]0.465
	T3-4	44 (91.7)	9 (81.8)	43 (84.3)	
N; n (%)	N0	22 (45.8)	6 (54.5)	25 (49)	[a]0.861
	N1-2	26 (54.2)	5 (45.5)	26 (51)	
M; n (%)	M0	27 (56.3)	5 (45.5)	36 (70.6)	[a]0.170
	M1	21 (43.8)	6 (54.5)	15 (29.4)	
Localization	Right	18 (37.5)	2 (18.2)	18 (35.3)	[a]0.368
	Left	18 (37.5)	7 (63.6)	26 (51.0)	
	Rectum	12 (25.0)	2 (18.2)	7 (13.7)	
Polyp	Present	19 (39.6)	3 (27.3)	14 (25.7)	[a]0.403
	Absent	29 (60.4)	8 (72.7)	37 (72.5)	
Differentiation	Well	3 (6.3)	3 (27.3)	7 (13.7)	[b]0.079
	Moderate	42 (87.5)	7 (63.6)	35 (68.6)	
	Poorly	3 (6.3)	1 (9.1)	9 (17.6)	
Number of metastatic sites	Single	13 (27.1)	4 (36.4)	14 (27.5)	[b]0.055
	Multiple	8 (16.7)	2 (18.2)	1 (2.0)	

[a]*Pearson'sChi-Square test* [b]*Fisher-Freeman-Halton Test* [c]*OnewayAnova Test*

Table IV: Comparison of pathologic features with mutational groups.

		KRAS	NRAS	RAS-wt	p-value
Lymphovascular invasion	Present	23 (47.9)	4 (36.4)	25 (49.0)	[a]0.742
	Absent	25 (52.1)	7 (63.6)	26 (51.0)	
Perineural invasion	Present	19 (39.6)	1 (9.1)	17 (33.3)	[a]0.155
	Absent	29 (60.4)	10 (90.9)	34 (66.7)	
Tumor budding	Low	25 (52.1)	8 (72.7)	30 (58.8)	[b]0.442
	Intermediate-high	23 (47.9)	3 (27.3)	21 (41.2)	
Crohn's-like reaction	Present	13 (27.1)	2 (18.2)	15 (29.4)	[a]0.749
	Absent	35 (72.9)	9 (81.8)	36 (70.6)	
Mucinous component	≤10%	34 (70.8)	10 (90.9)	42 (82.4)	[a]0.214
	>10%	14 (29.2)	1 (9.1)	9 (17.6)	
Signet ring cell	Present	1 (2.1)	0 (0)	2 (3.9)	[b]1.000
	Absent	47 (47.9)	11 (100)	49 (96.1)	
Solid pattern	Present	0 (0)	0 (0)	9 (17.6)	[b]0.003
	Absent	48 (100)	11 (100)	42 (82.4)	

[a]*Pearson'sChi-Square test* [b]*Fisher-Freeman-Halton Test*

DISCUSSION

This study evaluated the frequency of KRAS/NRAS mutations and clinicopathologic features of RAS mutant and wild-type CRCs in a cohort of 110 metastatic and non-metastatic patients.

KRAS and NRAS mutations in CRC were reported to be between 23% to 56% and 1% to 9% in the literature, respectively. KRAS exon 2 was the single most common mutation site comprising up to 88% of the RAS mutations [9, 12, 13]. Concordant with the previous results, mutation rates were 43.6% for KRAS and 10% for NRAS in this study. However; RAS mutations other than KRAS exon 2 were reflected 27.1% of the RAS mutations which was higher than the previously reported rates of 14% to 17% [4, 8, 14]. This high frequency of the non-KRAS exon 2 mutations identified in this study is probably due to the differences in the patients analyzed. Nevertheless; our results as others, emphasize the importance of extended RAS mutation analysis in mCRC as they also predict response to anti-EGFR therapy [4, 5, 14].

Up to date; KRAS/NRAS mutations have been associated with many patient and tumor-related factors [12, 15, 16]. Prior studies identified the correlation between NRAS (≥56) and KRAS mutations (>60 and >70 years) and the age of the patients [15-18]. However; the recent study of 1475 cases specifically investigating the correlation between distributions of mutations among age groups, did not reveal any association between patients' age and RAS mutations [13]. Also, there were controversial results reporting the correlation

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between KRAS/NRAS mutations and gender [18-20]. In line with other studies, our results did not show any correlation between patients' age and gender, and RAS mutational status. According to our findings family history and being a current/former smoker did not associate with KRAS/NRAS mutational status in CRC in accordance with Russo et al. In contrast, in a recent study including 3397 stage III CRCs, KRAS mutations found to be less likely to have a first degree relative with CRC and be a current/former smoker [16]. Recently; right and left sided colon carcinomas were identified as distinct entities in means of clinicopathologic features, molecular basis, and prognosis [21-23]. In general; the right sided tumors were more common in elderly patients and female sex. They harbor BRAF and KRAS mutations, and likely to have genome-wide hypermethylation via the CpG island methylator phenotype (CIMP) and microsatellite instability (MSI) pathways [21, 24]. Besides; NRAS mutations have been associated with the left sided and rectal carcinomas [15, 20]. Supporting this finding, 81.8% (9/11) of the NRAS mutated tumors were in the left colon and rectum in the present study, although this finding was statistically insignificant. The data investigating the association of lymph node involvement and distant metastasis with RAS mutation status demonstrated controversial results [9, 12, 13]. A few studies showed that KRAS mutations correlated with a higher frequency of lymph node metastases [25]. According to Peng et al., RAS mutations were more common in metastatic CRCs than non-metastatic CRCs [9]. Our results revealed an increased rate of RAS mutations in mCRC in comparison to non-metastatic CRC (67.2% vs 47.1%), but the result was statistically insignificant. Prior studies demonstrated that lung metastasis was more common in patients with KRAS mutant CRCs [10, 26]. Moreover, Yaeger et al. demonstrated that 2-year cumulative metastatic risk of KRAS/NRAS mutant CRCs to lung, brain, and bone was increased when compared to the RAS-wt tumors [27]. In this study, RAS-mutant tumors were associated with multiple organ metastasis in comparison to RAS-wt CRCs. However, a limitation of this study was distant metastasis other than liver, detected only in a small fraction of the cases. Therefore; it was not possible to compare mutation status with the metastatic site.

Following the previous studies lymphovascular and perineural invasion, signet ring cell/mucinous morphology, and MMR status did not show any difference between RAS mutational groups [12, 27]. Interestingly, none of the CRCs with solid growth pattern in the study cohort had RAS mutations. To the best of our knowledge, this has not been described previously. Regarding tumor grade, only a limited number of studies identified an association between KRAS mutation and well differentiated CRCs, whereas most of the literature could not reveal any relation [12, 16, 17]. Tumor budding emerged as an important additional prognostic factor in CRC patients in recent years. It is considered as an indicator of epithelial-mesenchymal transition and associated with adverse prognosis in malignant polyps and stage I-II patients [28]. Prall et al. have found that tumor budding was related to KRAS mutations [29]. In contrast, by the findings of this study, at least two other studies did not describe the association between tumor budding and Ras mutational status [27, 30].

STUDY LIMITATIONS

The present research has several limitations. First, the study is retrospective which may cause selection bias. Most importantly, our study lacked data on tumor CIMP and BRAF, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) status which may have a possible effect upon clinicopathologic features investigated. Finally, the study was conducted in a single community-based center and included a limited number of cases which may limit the generalization of the results and statistical power of the findings. An increased rate of non-KRAS exon 2 mutations was observed in the CRC of the Turkish population which may result from the inherent characteristics of the study cohort. As previously stated, NRAS mutant tumors were more frequently left-sided and RAS mutant tumors were associated with multiple organ metastases. To the best of our knowledge, this study described the absence of RAS mutations in CRCs with solid growth pattern for the first time in the literature. Other clinicopathologic characteristics of CRCs did not correlate with the RAS mutation status.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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